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Advanced analytical method of nereistoxin using mixed-mode cationic exchange solid-phase extraction and GC/MS

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ABSTRACT

Nereistoxin(NTX) was originated from a marine annelid worm Lumbriconereis heteropoda and its analogue pesticides including cartap, bensultap, thiocyclam and thiobensultap have been commonly used in agriculture, because of their low toxicity and high insecticidal activity. However, NTX has been reported about its inhibitory neuro toxicity in human and animal body, by blocking nicotinic acetylcholine receptor and it cause significant neuromuscular toxicity, resulting in respiratory failure. We developed a new method to determine NTX in biological fluid. The method involves mixed-mode cationic exchange based solid phase extraction and gas chromatography/mass spectrometry for final identification and quantitative analysis. The limit of detection and recovery were substantially better than those of other methods using liquid–liquid extraction or headspace solid phase microextraction. The good recoveries (97 ± 14%) in blood samples were obtained and calibration curves over the range 0.05–20 mg/L have R2 values greater than 0.99. The developed method was applied to a fatal case of cartap intoxication of 74 years old woman who ingested cartap hydrochloride for suicide. Cartap and NTX were detected from postmortem specimens and the cause of the death was ruled to be nereistoxin intoxication. The concentrations of NTX were 2.58 mg/L, 3.36 mg/L and 1479.7 mg/L in heart, femoral blood and stomach liquid content, respectively. The heart blood/femoral blood ratio of NTX was 0.76.

1. Introduction

Nereistoxin (NTX) is a natural toxic substance originating from a marine annelid worm, *Lumbriconereis heteropoda*, and it has strong insecticidal activity through blocking of the nicotinic acetylcholine receptor [1–4]. Several commercial insecticides such as cartap, bensultap, thiocyclam and thiosultap (Fig. 1) were developed from NTX and have been commonly used in agriculture due to their low mammalian toxicity and high effectiveness. However, subsequent studies have indicated that they pose potential toxicological risk to humans, not only for their acute toxicity, but also for their potential ontogenetic developmental toxicity, especially cartap [5–10]. These NTX-related insecticides are hydrolyzed to NTX in insects and in the human body [11]. The neurotoxicity of NTX in humans and animals has been reported. It can block the nicotinic acetylcholine receptor and also cause significant neuromuscular toxicity, resulting in respiratory failure. Bensultap and thiosultap were found to be degraded in water solution due to hydrolysis, even in the standard solution prepared in water [12]. In practice, cartap was also found to decompose in the gas chromatography column revealing an intensity of lower than one seventh to a sixtieth to that of NTX when its standard solutions were analyzed by direct injection using gas chromatography/mass spectrometry (GC/MS). Often the parent pesticides in water or biological fluids were not detected due to their degradation to NTX, also occurring during the processes of extraction and spectroscopic analysis. NTX is a precursor as well as an active metabolite and degradation product of the pesticides. When the concentration of NTX-analogous insecticides in biological fluids decreases greatly due to postmortem changes, detection becomes very difficult. Therefore, we attempted monitor NTX-analogous pesticides in forensic cases through analysis of NTX [11]. Since NTX is much more stable than the parent insecticides, reliable analytical results could be obtained, even at trace levels.

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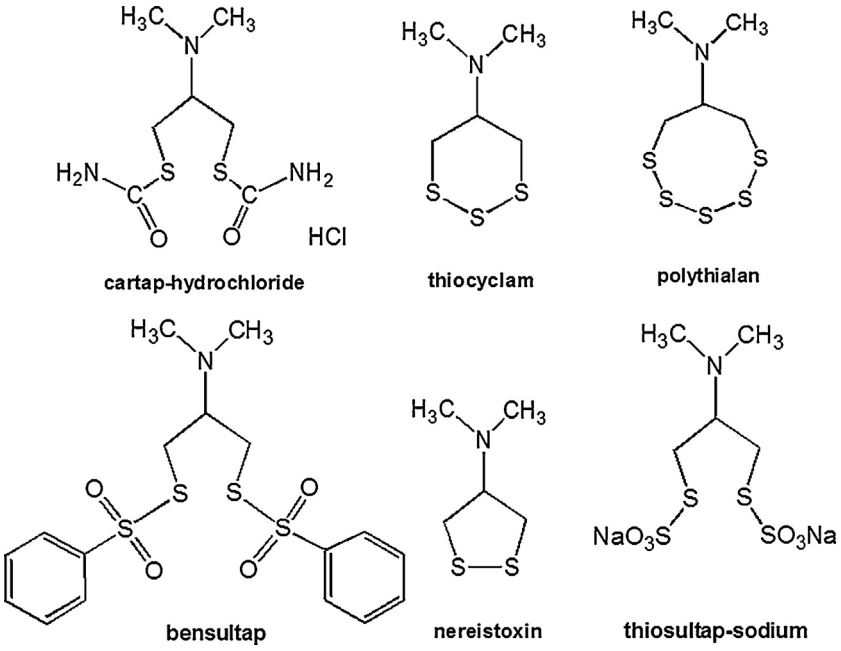

Fig. 1. The chemical structures of NTX-analogous insecticides.

A number of technologies such as GC/MS [13,14], liquid chromatography/mass spectrometry (LC/MS) [12] and fluorescent detection [15–17] have been established for the determination of these analogues and NTX in foods or biological specimens. For sample preparation, several procedures including liquid–liquid extraction (LLE) [11,15–17] headspace solid phase microextraction (SPME) [13] from bio-fluids and the QuEchERS® method [12] from foods and water samples have been introduced. However, the recoveries of NTX from blood samples using these preparation procedures was not sufficiently high due to moderate solubility of NTX to organic solvent and its volatile chemical property. When the concentration of NTX-analogous insecticides in biological fluids decreases greatly due to postmortem changes, the detection of target compounds becomes very difficult. For these reasons, a more reliable sample preparation procedure is required for the selective and good recovery of NTX from blood for analysis.

To achieve these goals, solid phase extraction (SPE) with a mixed-mode cationic exchange (MCX) cartridge was employed in this study. Oasis MCX® cartridges contain mixed-mode ion exchange polymer with hydrophobic and strong cation exchange capabilities (sulfonate group). Mixed-mode sorbents have been widely used for biological fluid analysis since they have two different chromatographic separation mechanisms, reversed phase and ion exchange [18–20]. The procedure for MCX SPE was optimized to maximize the selectivity and sensitivity to NTX by tuning the pH as well as changing the wash-elute solvent. The developed method was then applied for the detection and quantification of NTX in blood and gastric content samples collected from a cartap-related suicide case.

2. Materials and method

2.1. Reagent and chemical

Nereistoxin oxalate (98%) was purchased from Dr. Ehrenstorfer GmbH (Ausburg, Germany), and cartap hydrochloride (92%) was donated by Kyung-Nong Co. Ltd (Seoul, Korea). Sodium phosphate dibasic and sodium phosphate monobasic were of extra pure reagent grade, and were purchased from YAKURI pure chemicals Co. Ltd (Osaka, Japan). Methanol (MeOH) was of high performance liquid chromatography grade, and all other chemicals were of analytical grade or the highest purity available. All aqueous solutions were prepared in distilled water (DW) (18.2 MΩ cm) using Vivagen EXL-3® (Korea). Benzophenone, as an internal standard, was obtained from Sigma–Aldrich (USA). Mixed-mode cationic exchange (MCX®) cartridges were purchased from Waters Corporation (Wexford, Ireland).

2.2. Instruments

2.2.1. GC-MS

Sample analysis was carried out on an Agilent 7980A gas chromatograph with a DB-5MS capillary column (30 m × 0.25 mm i.d., film thickness 0.25 μm) and an Agilent 5975MSD mass spectrometer (CA, USA). The temperatures of the injection part and the MS interface were set to 270 °C and 280 °C, respectively. The oven temperature was held at 80 °C for 1 min, elevated to 180 °C at 20 °C/min and then to 290 °C at 30 °C/min, where it was held for 5 min for the total run time of 15 min. Samples were injected in splitless mode. The mass spectrometer was run in electron ionization (EI) mode with an electron energy of 70 eV.

2.3. Sample preparation

2.3.1. Standard stock solutions

Standard stock solutions of 100 mg/L for NTX and 1000 mg/L for cartap were dissolved in MeOH and stored at 4 °C in the refrigerator. Quantitative analysis was conducted to determine the NTX in the blood sample. Working solutions were prepared from stock solutions by dilution with DW and drug-free blood. Stock solution (100 mg/L) of benzophenone was prepared in MeOH. The benzophenone stock solution was diluted with water to the concentration of 10 mg/L for use as an internal standard.

2.3.2. Biological samples

Drug-free blood used for control and blank samples was obtained from forensic autopsy cases that were prescreened for drugs and alcohol. The blood samples were kept at 4 °C until analysis, and anticoagulant was not added. Whole blood samples